

Standard Operating Procedure

Depositional Benthic Invertebrate Sampling

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1 INTRODUCTION

1.1 Background

The Petite Ponar (Ponar) can be used for collecting benthic invertebrates from recently deposited surficial sediments. The Ponar's construction consists of a pair of stainless-steel weighted, tapered jaws that are held open by catch bars with a 6" x 6" opening and a minimum empty weight of 11 kg or 24 pounds (Figures 1 and 2). The upper portion of the jaws is covered with a steel mesh screen that allows water to flow through during descent, reducing the shock wave that precedes the sampler. The steel mesh screens are covered by flexible flaps that bend open from the flow during decent, but lay flat across the mesh during ascent to protect overlying fine sediments in the grab. During sampling, the Ponar is held open by a spring-loaded pin inserted into the catch bars. When the Ponar is not in use, the Ponar is held open by a safety pin (not spring-loaded) inserted into the catch bars.

Since benthic invertebrate sampling using a Ponar will disturb overlying waters, it should only be used after ambient water sampling has been completed. Do not disturb the substrate prior to Ponar deployment.

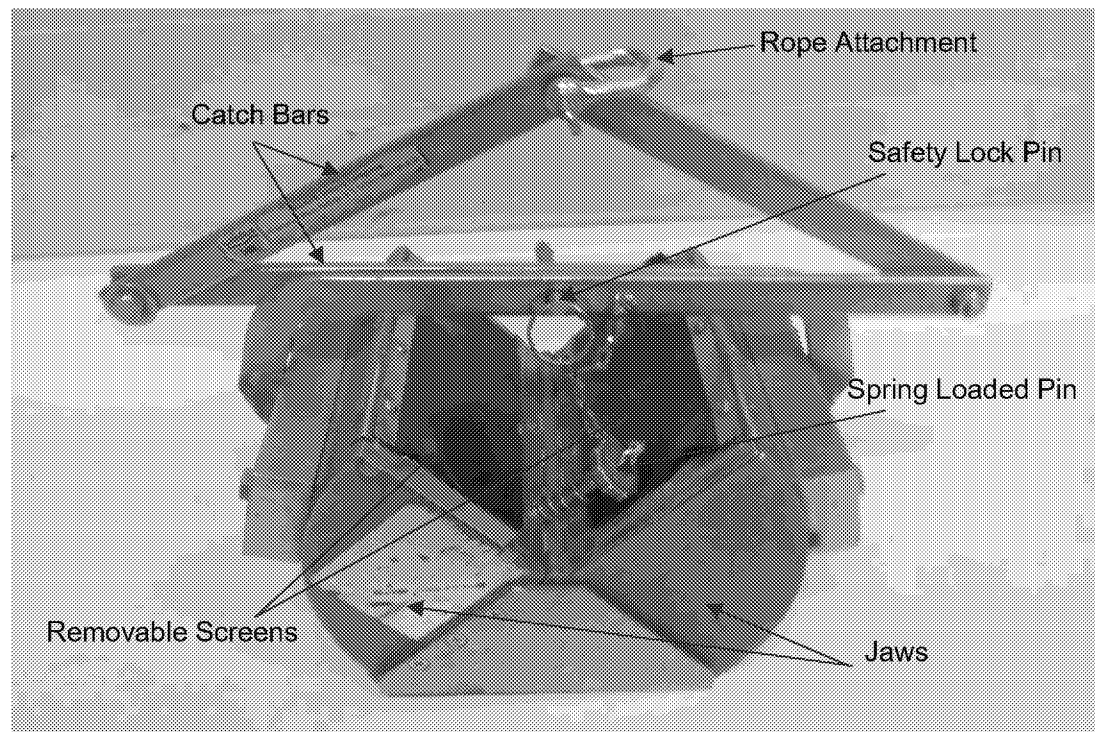


Figure 1: Open Ponar in "Safety Locked" Position



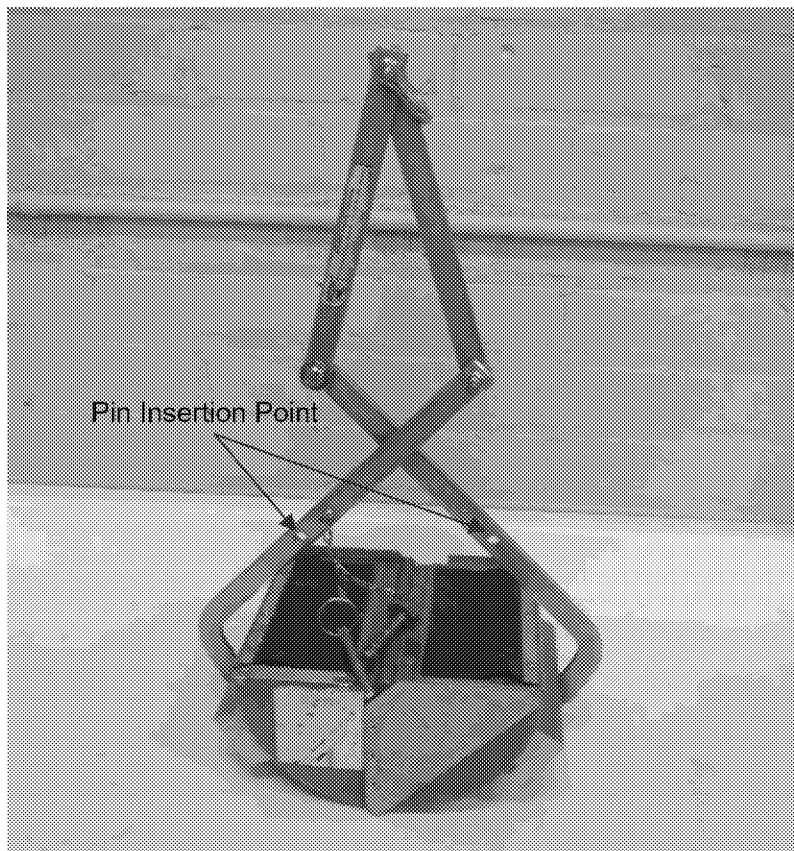


Figure 2: Closed Ponar in “Unlocked” or Closed Position

Note: This is how the sampler will look after grabbing a sediment sample.



2 BENTHIC INVERTEBRATE COLLECTION

2.1 Sample Collection

Obtain a benthic invertebrate sample using a Ponar as follows:

- a. Ensure that the Ponar jaws open and close properly and that the Ponar is clean and free of debris. Ensure that both the spring-loaded pin and the safety pin are tied to one of the catch bars as back-up in case the cable holding the pin in place breaks.
- b. Keep the Ponar jaws open by inserting the spring-loaded pin into the horizontal catch bars. Hold this pin in place by lifting the Ponar using the attached rope/cord, to maintain tension in the system. Particular attention must be paid to keeping hands away from the jaws and joints of the Ponar at this time.
- c. While standing or sitting in a boat at the benthic invertebrate sampling location, slowly and steadily lower the Ponar into the water on a rope. Do not lower the Ponar too quickly or allow it to free-fall, particularly as it approaches the bottom. The metal screens on top of the jaws can retard the flow of water if the sampler is lowered too quickly, creating a pressure wave that can force sediment away from the area of penetration and thus resulting in a non-representative sample. Steadily lowering the Ponar will also avoid triggering closure of the jaws before reaching the sediment.
- d. Once the Ponar is resting on the sediment surface, allow the rope to slacken to trigger release of the spring-loaded pin from the catch bars. The gravity-activated jaws will then “bite” into the sediment and start to close.
- e. Slowly raise the Ponar off the bottom to prevent loss of fine sediment while the Ponar closes and then raise it to the water surface.
- f. Upon recovery, remove sediment from the Ponar by first placing the Ponar in a shallow plastic tub and pressing down on the catch bars. Once the Ponar is open, insert the safety pin (not spring-loaded) into the catch bars to avoid accidental closure of the jaws.
- g. Lift the Ponar by holding onto the horizontal portion of the catch bars, so that the sediment will remain in the tub. Any sediment that is stuck to the inside of the Ponar can be removed by holding the Ponar over the tub and rinsing the insides of the Ponar using a strong stream of ambient surface water from a squirt bottle. Ensure all rinse water and sediment from the Ponar is collected in the tub below. Take care to rinse all internal surfaces including the screens.



- h. Evaluate the quality of the Ponar grab. Discard any rejected sample in such a way that it will not affect subsequent sampling efforts (e.g., discard on shore or into a separate container that can later be discarded once sampling in the area is complete). If the sample is rejected, start again at step b.; if it is accepted, continue to step i.
- Check for proper closure. If the jaws did not close completely due to lodged debris such as stones and pieces of wood, there will have been loss of material and therefore you must reject the sample.
 - Check the depth of penetration. If it was too shallow, invertebrates that dwell below the surface may have been missed, and the sample should be rejected.
 - Check to ensure the Ponar did not sample the sediment on an angle. If the sediment surface was not level in the grab, reject the sample.
- i. For each accepted grab, record the following information on the appropriate field sheet: water depth at sample location, approximate Ponar fullness (e.g., $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, full), presence of biological structures (e.g., macrophytes) and debris (e.g., wood chips) in sample, and odour (if present).
- j. Add water and mix sample gently to loosen clumps of sediment in the plastic tub. When sediments are loose, pour the contents into a nitex mesh sieve bag (500 μm , or as otherwise specified in the study design).
- k. Spray the plastic tub with a squirt bottle, washing all remaining sediment and benthic invertebrates into the mesh bag.
- l. Keeping the top of the mesh bag closed and above water, dunk the sieve bag in and out of the water repeatedly. This rinses away the sediment, leaving the benthic invertebrates behind. Continue rinsing until as much sediment as possible has been removed from the sample. In general, the water should run clear from the bag when all the sediment has been washed from inside the sieve bag.



3 BENTHIC INVERTEBRATE COMMUNITY COMPOSITION

3.1 Sample Collection

Collect the community sample as described in Section 2, then proceed as follows:

- a. Once the sample has been thoroughly rinsed, transfer all contents of the mesh bag into a pre-labelled sample container by spraying the mesh with a squirt bottle. Work over a larger plastic tub to ensure no sample is lost during transfer.
- b. Turn the mesh bag inside out, and use tweezers to remove any invertebrates that may still be clinging to the mesh.
- c. Affix the lid and take the sample to an appropriate location for preservation (See Section 5). In addition to labelling the outside of the sample jar, a liquid-proof internal label (e.g., pencil or marker on water proof paper or Popsicle stick) must be inserted into the sample jar during preservation.
- d. Preserve the sample in a 10% buffered formalin solution (i.e., powdered borax and formaldehyde) equivalent to 10% of the sample volume.
 - Formalin is a carcinogen and an irritant to workers, so protective gloves, fit tested respiratory gear, and eye protection are required.
 - Preserve the sample as soon as practical after sampling to prevent predatory invertebrates from preying on others in the samples. After adding the preservative, gently mix the sample several times to ensure that the preservative has thoroughly penetrated the sample. Samples can be kept at room temperature following preservation.
 - Ship samples preserved in formalin to the analytical laboratory within 3 weeks of collection to prevent acidic degradation of organisms by the formalin (i.e., the taxonomy lab will remove the formalin preservative and replace it with 70% ethanol to avoid decalcification of organisms).
 - Always ship samples by ground transportation (not air).

Containers used for benthic invertebrate community samples must:

- a. Be large enough to have sufficient space remaining for the addition of preservative;
- b. Be leak-proof and sturdy enough for routine handling and transportation;



- c. Have physical and chemical properties that are unaffected by the fixative/preservative;
and
- d. Conform to regulations concerning the transportation of dangerous goods.



4 BENTHIC INVERTEBRATE TISSUE CHEMISTRY

4.1 Sample Collection

Collection of benthic invertebrates for tissue chemistry analysis should be completed after the community composition sample has been taken. These samples are not preserved in formalin but are picked free of debris in the field, with live invertebrates being frozen following collection. Collect the tissue sample as described in Section 2, then proceed as follows:

- a. Once the sample has been thoroughly rinsed, transfer all contents of the mesh bag into a white plastic tub by turning the bag inside-out and spraying the mesh with a squirt bottle. Ensure that no sample is lost during transfer.
- b. Use tweezers to remove any invertebrates that may cling to the mesh.
- c. Locate and pick all visible organisms and place them into labelled sterile cryovials, removing as much water as possible. Ensure that:
 - All benthic invertebrates are alive when collected.
 - The sample contains only benthic invertebrates (no other organic/inorganic matter).
 - A squirt bottle can be used to remove sediment and detritus from benthic invertebrates prior to adding them to the sample container.
 - Benthic invertebrates that use exogenous materials for their cases must be separated from them before they are added to the sample (e.g., caddisflies are to be separated from their cases and the cases discarded).
- d. If the desired sample size (e.g., 2 g of tissue) is not reached with the prescribed number of grabs, additional grabs may be required. Record any additional effort on field sheets.
- e. Keep the samples in a cooler with ice packs until transfer to a freezer later in the day. At the end of the field program, ship samples by ground transportation (not air), ensuring that there is sufficient ice in the cooler that samples reach the laboratory frozen.



5 FROM THE 2018 TO 2020 KOOCANUSA STUDY DESIGN

5.1 General Information

Benthic invertebrate community samples will be collected in August 2018 at the five stations located upstream and downstream of the Elk River. Benthic invertebrate tissue samples will be collected twice annually in the 2018 to 2020 monitoring program (April and August) with samples collected in the same areas as community samples, both upstream and downstream of the Elk River.

5.2 Sample Collection

5.2.1 Community

Consistent with the 2014 to 2016 study, benthic invertebrate community sampling will be completed at each of five stations upstream and downstream from the Elk River in August 2018, when water levels are most stable and benthic invertebrate communities are anticipated to be at peak biomass and diversity. Benthic invertebrate community samples will be collected using a stainless steel Petite Ponar sampler. A single sample, consisting of a composite of five Petite Ponar grabs, will be collected at each station with care taken so that each grab captures the surface material and is full to each edge. Incomplete grabs will be discarded. Each acceptable grab will be field-sieved using 500 µm mesh with the retained material carefully transferred into a plastic sampling jar containing both external and internal station identification labels. Benthic invertebrate samples will be preserved to a level of 10% buffered formalin in ambient water and submitted to a certified benthic taxonomist for analysis.

5.2.2 Tissue Chemistry

A single composite benthic invertebrate tissue sample will be collected upstream and downstream of the Elk River in April and August of 2018 to 2020. A total of 20 Petite Ponar grabs (four from each of the five sampling stations in each study area) will be collected to obtain a single sample. Each grab will be placed into a 500 µm mesh sieve bag and sieved free of material less than 500 µm in size. The remaining material will be transferred to a white enamel tray for removal of benthic organisms using tweezers. Visible organisms will be removed from the debris/sediment and rinsed clean using ambient water. Similar to sampling conducted in 2014 to 2016, chironomids will be targeted for tissue collection, but if chironomids are not present in sufficient numbers, other benthic invertebrates will be added to the sample (and noted on field sheets) to achieve sufficient sample weight for analysis (minimum of 0.5 g).



5.3 Laboratory Analysis

5.3.1 Community

Benthic invertebrate community analysis will follow standard sorting methods which incorporate recommended quality assurance/quality control procedures for assessing sub-sampling error and sorting recovery checks (Environment Canada 2012¹). Upon arrival at the laboratory, a biological stain will be added to each sample to facilitate greater sorting accuracy. Samples will be washed free of formalin in a 500 µm sieve and examined under a stereomicroscope at a magnification of at least ten times. Benthic invertebrates will be removed from the sample debris and placed into vials containing a 70% ethanol solution according to major taxonomic groups (e.g., phyla, orders). A senior taxonomist will enumerate and identify benthic organisms to the lowest practical level (typically to genus or species) using the most recent taxonomic keys. Following identification, representative specimens of new taxa will be preserved in a 75% ethanol, 3% glycerol solution in separately labelled vials and added to the voucher collection for the project.

5.3.2 Tissue Chemistry

Benthic invertebrate tissue samples will be promptly shipped to a qualified laboratory for analysis of metals (including mercury) and selenium using high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). The laboratory will freeze dry the samples prior to analysis. Concentrations will be reported on a dry weight basis, along with moisture content to allow conversion to wet weight values, as required. Accuracy and precision of laboratory data will be judged based on ability to achieve minimum laboratory reporting limits, as well as replicate analysis (minimum of 10% of samples) and comparison to certified reference materials.

¹ Environment Canada. 2012. Metal Mining Technical Guidance for Environmental Effects Monitoring. ISBN 978-1-100-20496-3.

